

ABSTRACT

SPECTRAL CALIBRATION OF FLUORESCENT
POLYNUCLEOTIDE SEPARATION APPARATUS

The invention relates to methods, compositions, and systems for calibrating a fluorescent polynucleotide separation apparatus. One aspect of the invention is multiple color calibration standards and their use. A multiple color calibration standard is a mixture of at least two polynucleotides of different length, wherein each of the polynucleotides is labeled with a spectrally distinct fluorescent dye. Another aspect of the invention is to produce total emission temporal profiles of multiple color calibration standards for use in calibrating fluorescent polynucleotide separation apparatus. The peaks corresponding to the fluorescently labeled polynucleotides in the total emission temporal profile may be detected using a peak detector that is driven by changes in the slopes of the total emission temporal profile. Calibration of fluorescent polynucleotide separation apparatus, with various embodiments of the methods of the invention, includes the step of identification of the labeled polynucleotides of the multiple color calibration standards. The process of spectral calibration of a fluorescent polynucleotide separation apparatus using a multiple color calibration standard may include the step of the estimating (extracting) of the dyes' reference spectra, using information from the peak detection process performed on the total emission temporal profile. Other aspects of the invention include systems for separating and detecting fluorescently labeled polynucleotides, wherein the system is designed for spectral calibration in accordance with the subject calibration methods employing multiple color calibration standards. Another aspect of the invention is methods and compositions for detecting the flow of electrical current through a separation channel of a fluorescent polynucleotide separation apparatus. These methods and compositions employ monitoring dyes. Monitoring dyes are fluorescent dyes that are spectrally distinct from the dye on the polynucleotide intended to convey genetic information, e.g., fluorescent polynucleotide sequencing reaction products.